PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P810PC00	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/DK2004/000659	International filing date *(day/month/year)* 29.09.2004	Priority date *(day/month/year)* 30.09.2003
International Patent Classification (IPC) or national classification and IPC C07K5/00, C07K7/00, C07K14/00, G01N33/68, A61K38/04, A61K38/17		
Applicant ENKAM PHARMACEUTICALS A/S et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 11 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 13 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☒	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
26.08.2005	20.03.2006

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/DK2004/000659

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-81, 86-90	as originally filed
82-85	received on 29.08.2005 with letter of 26.08.2005

Sequence listings part of the description, Pages

1-23	as originally filed

Claims, Numbers

1-41	received on 20.02.2006 with letter of 17.02.2006

Drawings, Sheets

1/63-55/63, 57/63, 59/63-63/63	as originally filed
56/63, 58/63	received on 29.08.2005 with letter of 26.08.2005

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/DK2004/000659

Box No. II Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
   - ☐ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
   - ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).

2. ☒ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

   see separate sheet

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1-6, 40 completely; 8-39 and 41 partially

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☒ the claims, or said claims Nos. 8-39 and 41 (partially) are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-6 and 40 (completely); 8-39 and 41 (partially) concerning inventions 2-4

   ☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

   ☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

   ☒ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation to restrict or pay additional fees, the applicant has:
   - ☐ restricted the claims.
   - ☐ paid additional fees.
   - ☐ paid additional fees under protest.
   - ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 8-39 and 41 (partially), concerning invention 1 .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	8-39 and 41 (partially)
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	8-39 and 41 (partially)
Industrial applicability (IA)	Yes:	Claims	8-39 and 41 (partially)
	No:	Claims	

2. Citations and explanations (Rule 70.7):

   see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Reference is made to the following documents:

D1: RAO Y ET AL: "Identification of a peptide sequence involved in homophilic binding in the neural cell adhesion molecule NCAM" JOURNAL OF CELL BIOLOGY, ROCKEFELLER UNIVERSITY PRESS, NEW YORK, US, US, vol. 118, no. 4, August 1992 (1992-08), pages 937-949

D2: DATABASE HTTP://WWW [Online] 2002, KASPER ET AL.: "Extracellular modules of the cell adhesion molecules", retrieved from HTTP://WWW-HASYLAB.DESY.DE/SCIENCE/ANNUAL_ REPORTS/2002_REPORT/PART2/CONTRIB/72/7824. PDF

D3: ATKINS A R ET AL: "Solution structure of the third immunoglobulin domain of the neural cell adhesion molecule N-CAM: can solution studies define the mechanism of homophilic binding?" JOURNAL OF MOLECULAR BIOLOGY, LONDON, GB, vol. 311, no. 1, 3 August 2001 (2001-08-03), pages 161-172

D4: RONN L C B ET AL: "IDENTIFICATION OF A NEURITOGENIC LIGAND OF THE NEURAL CELL ADHESION MOLECULE USING A COMBINATORIAL LIBRARY OF SYNTHETIC PEPTIDES" NATURE BIOTECHNOLOGY, NATURE PUBLISHING, US, vol. 17, October 1999 (1999-10), pages 1000-1005

D5: SOROKA VLADISLAV ET AL: "Induction of neuronal differentiation by a peptide corresponding to the homophilic binding site of the second Ig module of the neural cell adhesion molecule" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 277, no. 27, 5 July 2002 (2002-07-05), pages 24676-24683

D6: KRISTIANSEN L V ET AL: "Homophilic NCAM interactions interfere with L1 stimulated neurite outgrowth" FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 464, no. 1-2, 24 December 1999 (1999-12-24), pages 30-34

D7: JENSEN PETER HOLME ET AL: "Structure and interactions of NCAM modules 1 and 2, basic elements in neural cell adhesion" NATURE STRUCTURAL BIOLOGY, vol. 6, no. 5, May 1999 (1999-05), pages 486-493, XP002315063 ISSN: 1072-8368

D8: KASPER CHRISTINA ET AL: "Structural basis of cell-cell adhesion by NCAM" NATURE STRUCTURAL BIOLOGY, vol. 7, no. 5, May 2000 (2000-05), pages 389-393

D9: WO 00/18801 A2 (ROENN, LARS, CHRISTIAN, B; BOCK, ELISABETH; HOLM, ARNE; OLSEN, MARIANN) 6 April 2000 (2000-04-06)

D10 Huang et al. Biopolymers **43** (1997) 367-382

Re Item III
Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Present **claims 8-39 and 41** partially relate to an extremely large number of possible uses of compounds, methods and compounds per se. In fact, the claims contain so many options and variables, that a lack of clarity (and conciseness) within the meaning of Article 6 PCT arose to such an extent that a meaningful full search of the claims was rendered impossible.
Consequently, the search was and therefore also this opinion is restricted to those parts of the application which do appear clear and concise, namely the compounds and methods of **invention 1** when referring to polypeptides with specified sequences (**SEQ ID NOs: 1-3, 40 and 41**), and not to undefined fragments variants thereof.

Re Item IV
Lack of unity of invention

The separate inventions/groups of inventions are:

Invention 1: Claims 8-39 and 41, partially
Use of compounds, capable of binding to the NCAM homophylic binding site composed of the Ig1, Ig2 and Ig3 modules and thereby modulating the interaction between Ig1 and Ig3 modules from two individual NCAM molecules; methods of modulating cells presenting NCAM.
Invention 2: Claims 8-39 and 59, partially
Use of compounds, capable of binding to the NCAM homophylic binding site composed of the Ig1, Ig2 and Ig3 modules and thereby modulating the interaction between Ig2 and Ig3 modules from two individual NCAM molecules; methods of modulating cells presenting NCAM.
Invention 3: Claims 8-39 and 41, partially
Use of compounds, capable of binding to the NCAM homophylic binding site composed of the Ig1, Ig2 and Ig3 modules and thereby modulating the interaction between two Ig2 modules from two individual NCAM molecules; methods of modulating cells presenting NCAM.

Invention 4: Claims 1-6 and 40
Crystals of a polypeptide comprising the Ig1-Ig2-Ig3 module of NCAM, their use and method of crystallisation.
Invention 5: Present Claim 7 completely
Method for selecting a candidate compound based on a structural model of the Ig1-Ig2-Ig3 modules of NCAM, obtainable eg from the soluble or crystalline polypeptide.

They are not so linked as to form a single general inventive concept (Rule 13.1 PCT) for the following reasons:

Introduction:
Two structurally related CAMs, the neural cell adhesion molecule (NCAM) and L1, are prominent members of the immunoglobulin superfamily, and are also known to interact with each other (Kristiansen et al. 1999; D6). Recombinant Ig modules 1, 2 and 3 of NCAM, involved in homophylic NCAM binding (see abstract of **D6**), gave complete inhibition of L1 induced neurite outgrowth. NCAM engages also in a calcium-independent, homophilic binding originally suggested to depend on a reciprocal interaction between the third Ig-module, or on all five Ig-modules of two opposing NCAM molecules; later it has been shown that also the first and the second Ig-modules of NCAM bind to each other in a so-called double reciprocal interaction (eg Atkins et al. Fig. 1; **D3**). Using NMR spectroscopy the 3D-structure of the first and second Ig-module of NCAM was recently solved, and putative reciprocal binding sites were identified, providing a structural model of an anti-parallel binding between the two Ig-modules (Jensen et al.; **D7**); crystallisation and structural data of high quality crystals of NCAM Ig1-Ig2 were provided by Kasper et al. ((2000); **D8**).

Motivation for the split into five inventions:
In the present invention, the structural work has been extended (see Kasper et al. (2002); **D2**) in comparison to **D8** by elucidating the 3D structure of the Ig1-Ig2-Ig3 module of NCAM; D2 mentioned already the crystallization of the Ig1-2-3 triple-domain and the importance of Ig3 in homophylic binding (see also Soroka et al (2000), **D5**, in particular the introduction when citing references 5 and 6). The solution structure of the Ig3 module had already been disclosed in **D3**, as well as the expression of recombinant chicken IgI-III NCAM and a mutant (Phe19) thereof, establishing a residue important in Ig1-2

dimerization. 3D structural studies can be standardly carried out, eg as described earlier in the prior art to find parts of the modules interacting with each other, and to propose compounds interfering with the contact points; in addition, the model can be used to evaluate the binding of peptides known to be involved in homophylic binding (e.g. peptide P5 disclosed by Rao et al. (**D1**), derived from chicken Ig3 and with sequence KYSFNYDGSELIKKVDKSDE (see Table III), has already been referred to in relation to modulation of NCAM homophylic binding; this peptide, as part of chicken Ig3, has the corresponding sequence SEQ ID NO:20 of rat Ig3 as presently mentioned in the description (see Figure 11 of D1); in **D5** a peptide P2 derived of the Ig2 module is disclosed, P2 with sequence GRILARGEINFK (see eg Figure 9), being involved in Ig1 binding, neurite outgrowth and inhibiting cell aggregation (see also **WO 00/18801**, SEQ ID NO:23); in **D4** (Ronn et al.) a combinatorial library was used to find a synthetic, neuritogenic peptide C3, with sequence ASKKPKRNIKA, binding to Ig1 at a site different from the binding site of the NCAM Ig2 module; see also WO 00/18801, SEQ ID NO:1). WO 00/18801, in particular page 24 line 18 and further, discloses SEQ ID NO:26 with sequence GEISVGESKFFL, an Ig1 peptide binding apparently to the part of the homophilic binding site of NCAM Ig1-Ig2 which is constituted by the Ig2 domain and identical to SEQ ID NO:19 of the present application.

Thus a method of modulating outgrowth of neurites presenting NCAM with different NCAM ligands interacting with homophylic binding of NCAM, in particular involving the Ig1 and Ig2 modules,was already known, as well as crystals and structure of the Ig1-Ig2 fragments of the cross-like, anti-parallel Ig1-Ig2 dimer (Kasper et al 2000); furthermore, the solution structure of the Ig3 module had been disclosed as well as the role of Ig3 in homophilic binding. The crystallisation of Ig1-Ig2-Ig3 has been suggested and different peptides were known to interfere with homophilic binding (reference is made to the known SEQ ID NO:19 as referred to in the present application), as well as methods to find additional peptide sequences (by rational design based on structure or by combinatorial libraries).

Conclusion:

It is therefore considered that a special technical link between the inventions I-III, the crystals of Ig1-Ig2-Ig3 or selection methods is absent. According to Rule 13 PCT, a group of inventions is only linked to form a single inventive concept where there is a technical relationship among the inventions that involves at least one common or corresponding

special technical feature that defines the contribution which each claimed invention, considered as a whole, makes over the prior art. No such a technical relationship for the listed five inventions is identifiable in view of the cited prior art with respect to the structural studies to obtain of the first three NCAM modules and the peptides relevant to several types of NCAM homophylic binding. Accordingly, the claims of these five inventions are not so linked by a special, new and inventive technical feature under PCT Rule 13 and therefore lack unity of invention is present.

To be noted is that further non-unitarily linked subject-matter appears to be present within present invention 1 on the basis of the fact that SEQ ID NO:20 was obvious to the skilled person. Each specified peptide and its use as a ligand appears therefore to represent a separate invention.

The applicant decided to pay one additional search fee under protest with respect to invention 5. After the Chapter II request, the Applicant was requested to either limit the application to invention 1 or 5, or to pay a further examination fee for the second searched invention. The Applicant decided not to answer to this invitation, and the **IPER** (International preliminary examination report) is therefore established **for the first invention only.**

Re Item V
Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Newly filed claim 29 has been amended: however, it is noted that this claim is not considered to be of a second medical use-type, as it does not specify a particular **medical therapy** for which the manufactured medicament will be of use; it only specifies which cells the compound should modulate.

2. The present invention does not satisfy the criterion set forth in Article 33(3) PCT because the subject-matter of claims 8-39 and 41 (as far as invention 1 is concerned) does not involve an inventive step (Rule 65(1)(2) PCT).

   The peptides of claim 8, considered to belong to invention 1 and partially searched

(the claim has an undefined scope by referring to "a fragment or a variant of said sequence"), are for example the peptides having SEQ ID NO:40 and 41 (being a part of Ig1; present claims 27-28). The other sequences belonging to invention 1 have been submitted to be SEQ ID Nos 1-3 (description page 84, last paragraph).

3. The peptides having sequences like SEQ ID NO:40 and 41 are considered to have been obvious to the skilled person in view of the combination of documents **D2** (see the top of page 2) and **D3.** The consideration of peptide sequences with respect to binding sites follow in an obvious way from the 3D-structure. At present, it has to be noted that nothing indicates that the skilled person was not in the position to repeat the crystallisation indicated in D2; with respect to D3 it is noted that this document leaves several options open with respect to the interacting Ig domains, and it concludes (in the abstract) that in solution different interactions are possible than that occur on the cell surface, *eg* the interactions in crystals may come closer to the true domain interactions. The reasoning about obviousness applies also to the pharmaceutical use, as this use was already suggested in the prior art for this type of peptides. Said last mentioned peptides appear also to lack the right of priority, making the **P,X document** of the search report (the publication of the present invention) available as a citable document.

4. With respect to the peptides with sequences SEQ ID NO:1 and 2 (part of Ig1) and SEQ ID NO:3 (part of Ig3), it is additionally noted that these peptide have not been demonstrated to **bind** to a NCAM homophylic binding site composed of Ig1/Ig3 modules of NCAM. It is therefore not clear if the technical problem is likely to be solved for these peptides. This demonstration is necessarry for the acknowledgement of the inolvement of an inventive step.

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